INFLUENCE OF THE IONS OF TRIVALENT IRON ON THE PHYSICAL-CHEMICAL PROPERTIES OF SOLUTIONS OF NATIVE DESOXYRIBONUCLEIC ACID

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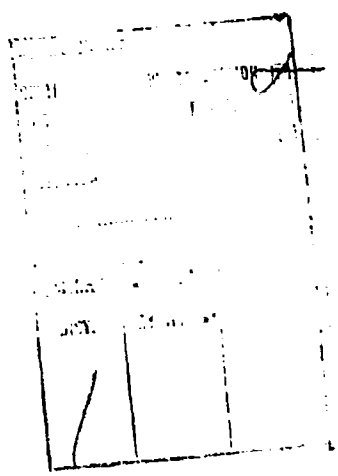

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INFLUENCE OF THE IONS OF TRIVALENT IRON ON THE PHYSICAL-CHEMICAL PROPERTIES OF SOLUTIONS OF NATIVE DESOXYRIBONUCLEIC ACID

[Following is the translation of an article by Ilina, A. N., Zhdanova, V. D., Moshkovskiy, Yu. Sh. and Mirlina, S. Ya., Institute of Chemical Physics, USSR Academy of Sciences, Moscow, appearing in the Russian-language periodical Biofizika (Biophysics), Vol X, No 6, 1965, pages 929--934. It was submitted on 8 Apr 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

In the work conducted by Wacker and Vallee [1] it was shown that all preparations of RNA and DNA, isolated from various sources using the necessary precautions, contained quite large quantities of atoms of various metals: Mg, Ca, Sr, Ba, Al, Cr, Mn, Fe, Ni, Cu, Zn.

The total amount of all attendant metals, expressed in g · atom/mole of phosphate of RNA, corresponded to one atom of metal per 50 nucleotides. It is interesting to note that RNA and DNA, isolated from various sources, contained the relative amount of metals which was characteristic for the given specimen. Many metals, for example -- iron, are so solidly bound with the macromolecule of nucleic acid that even the strongest complex forming substances are not capable of eliminating them.

Thus, in the accepted structure of nucleic acid, which is made up of nitrogen bases, and a balance of sugar and phosphate, it is also necessary to include atoms of metal.

It is not excluded that the atoms of the metals which are included in the structure of nucleic acids play a significant biological role. For an understanding of the role of metals which are solidly bound with DNA and RNA, it is necessary to study how the metals influence the physical-chemical properties of the macromolecule, and also the structures of the complexes which are formed.

Methods and Materials

The samples of DNA were obtained from the thymus of a calf by the phenol method according to Kirby [2] with subsequent deproteinization by the method of Georgiev [3]. The protein content was determined by the method of Lowry [4] and did not exceed 1--2%. The value Ep = 6700 and the hypochromic effect, equal to 1.34--1.36, testified to a sufficient degree of the original state of the DNA specimens. For the formation of complexes an aqueous solution of $FeCl_3$ (dry salt of the grade "kh.ch.") was used in a

quantity of from 0.1 up to 0.5 M per 1 mole of nucleotides. /Probable meaning of kh. ch. -- khimicheski chistyy or chemically pure./

For obtaining the dependency curves of the value of the viscosity presented $\left(\frac{\eta_{sp}}{c}\right)$ on the concentration of DNA we used both the high gradient Ubbelohde viscosimeter with a velocity gradient $G = 1600\ sec^{-1}$, and also the special low gradient Ostwald viscosimeter with three spheres, which is described in the work. The values of the velocity gradients of the latter were $G_1 = 72.0\ sec^{-1}$, $G_2 = 45\ sec^{-1}$, $G_3 = 39.6\ sec^{-1}$.

The absorption spectra in the ultraviolet range were taken on a SF-4 spectrophotometer, in special thermostatic cuvettes with a layer thickness of 1 cm. The temperature in the cuvette was measured with a thermocouple and maintained constant with an accuracy of $\pm$ 0.1°C.

The dispersion of optical rotation (DOR) was measured in the range of 550--265 mμ in cylindrical cuvettes 5 cm thick on the domestic "Polimer" spectropolarimeter. The temperature in the cuvettes was maintained constant with an accuracy of $\pm$ 0.2°C.

Results and Discussion

The measuring of viscosity in the high gradient Ubbelohde viscosimeter was carried out with solutions of DNA in 0.15 NaCl at concentrations of 0.3--1.3 mg/ml. With such large concentrations of DNA the intermolecular reaction may exert an influence on the value of the $\frac{\eta_{sp}}{c}$. Besides this, there is a possibility of the formation of stable macromolecule aggregates with the addition of ions of Fe^{3+}, if the latter create coordination valences between the neighboring molecules of the biopolymer.

Figure 1 presents the dependency curves of $\frac{\eta_{sp}}{c}$ on the concentration for solutions of native DNA and DNA with a various amount of $FeCl_3$, obtained on the Ubbelohde viscosimeter with $G = 1600\ sec^{-1}$. The amount of addend iron salt was limited by the precipitation of the DNA, setting in at a relationship of around 0.5 M $FeCl_3$ per mole of nucleotide.

As is seen from figure 1, the dependency curves of $\frac{\eta_{sp}}{c}$ on the concentration obey the Huggins equation. The addition of the ions of Fe^{3+} lead to a noticeable lessening of the value of $\frac{\eta_{sp}}{c}$, and also to a lessening of the value of the Huggins constant. These results show that in the range of concentrations of Fe^{3+} ions which was used, a noticeable aggregating of macromolecules of DNA did not take place and, consequently, the formation was not observed of intermolecular coordination valences of Fe^{3+} with the macromolecules of DNA.

An analogous influence of Fe^{3+} ions on $\frac{\eta_{sp}}{c}$ was obtained on the low gradient viscosimeter in a range of concentrations of 0.002--0.006 g/100 ml. The selected range of concentrations reduced the intermolecular reaction by a significant degree.

As is seen from figure 2, the curves of $\frac{\eta_{sp}}{c}$ versus the concentration in the case of a low gradient viscosimeter do not obey the Huggins equation, but with the addition of Fe^{3+} ions, just as in the previous case, a lessening of viscosity takes place. The detected reduction of the viscosity values following the addition of Fe^{3+} may be explained either by the denaturing action, which leads to the formation of a molecule of DNA which is coiled into a ball, or by the still greater straightening of the native macromolecule. In the absence of Fe^{3+} ions the native macromolecule of DNA should have a certain number of points, around which there is the possibility for the turning of components of the molecule which are stable rods, according to the model of Watson and Crick /5/, relative to each other.

A similar model was proposed by Sadron /6/ without any experimental basis. To make a selection between the above mentioned alternative models just on the basis of viscometric measurements was not possible. Therefore, investigations were conducted on the influence of adding Fe^{3+} ions on the melting curves and on the dispersion curves for the optical rotation of DNA, thus producing information on the degree of the native state of the DNA structure.

When measuring the DOR of native DNA and complexes of DNA with Fe^{3+}, dependency curves were obtained for the specific optical rotation $[\alpha]$ on the wave length. As is seen from figure 3, with an increase in the content of Fe^{3+} there takes place an increase in the value of $[\alpha]$, while the position of the maximum remains unchanged. This result testifies in favor of the proposal that the addition of Fe^{3+} ions to a solution of DNA leads to an increase in the number of helical sectors in the macromolecule.

This is also in agreement with the data from the spectrophotometric investigation of solutions of pure DNA and complexes of DNA with Fe^{3+}. An increase in the concentration of Fe^{3+} ions causes a distinct reduction in the values of the optical density of solutions of DNA. According to the contemporary supposition concerning the nature of the hypochromic effect in helical polymeric molecules, a lessening of the absorption coefficient of a solution of DNA is related to the greater orderliness of nitrogenous bases, which should take place with an increase in the proportion of helical sectors.

The formation of a complex of DNA with Fe^{3+} in 0.15 M NaCl exerts a noticeable influence on the melting curves, obtained when measuring the dependency $[\alpha]$ and optical density on the temperature. (figure 5). In the case of a melting curve, taken according to the dispersion of optical rotation, the temperature of melting for native DNA equaled 77°, and for the complex of DNA with Fe^{3+} it was 83°.

Closer values for the melting temperatures were obtained (81° for pure DNA and 83° for DNA with Fe^{3+}) when the fusion curve was taken according to the optical density.

The question arises: Which structural model can explain the demonstrated influence of the ions on the viscosity and optical properties of solutions of DNA? It may be suggested that the model of the double helix of

DNA, proposed by Watson and Crick, is not realized over the entire extent of the gigantic macromolecule, but only in specific sectors which are connected with each other by places in which it is possible for the sectors to turn relative to each other, as this is schematically shown in figure 6, A. Such places may be ruptured in one of the two chains of the DNA helix, thus permitting the neighboring sector to be turned at somewhat of an angle. This leads to the fact that only part of the sectors will be oriented along the flow of the fluid. With the addition of Fe^{3+} ions to the solution of DNA their bonding takes place.

There may be various groups at the sites of bonding of the Fe^{3+} ions, such as phosphate groups or nitrogenous bases. The sites of breaks in one of the chains of DNA may turn out to be very active in respect to the ions of Fe^{3+}. Here it may be possible to consider the formation of coordination valences between the two ruptered chains and the Fe^{3+} ion (figure 6, B). This leads to a loss of freedom of rotation for the sectors and to a greater rigidity of the macromolecule. Apparently, in this case the orientation of the molecules of DNA in the flow will be significantly better than in solutions of pure DNA. Such a proposal concerning the structure of a complex of DNA with Fe^{3+} conforms with the decrease in the quoted viscosity $\frac{\eta_{sp}}{c}$ with the addition of Fe^{3+} ions.

A similar model of the structure of the complex also agrees well with the results of optical investigations, which showed that the addition of Fe^{3+} ions leads to an increase in the portion of helical sectors in a molecule of a biopolymer.

Conclusions

1. An investigation was made on the influence of the addition of Fe^{3+} on the viscosity, dispersion curves of optical rotation and the absorption spectra of solutions of native DNA.

2. It was shown that with the formation of a complex of DNA with Fe^{3+} a lessening takes place in the values of the quoted viscosity in a wide range of concentrations of DNA.

3. In the presence of Fe^{3+} ions an increase is observed in the value of the specific rotation and a decrease in the absorption coefficient of solutions of DNA, and also an increase of the melting temperature in comparison with the melting temperature of pure DNA.

4. The experimental data obtained agree with the model of a macromolecule of DNA, having a certain number of points at which there is the possibility for the rotation of parts of the molecule which are stable rods. The addition of Fe^{3+} ions leads to an increase in the rigidity of the DNA macromolecule, by reducing the number of places around which a bending of the macromolecule takes place.

Literature

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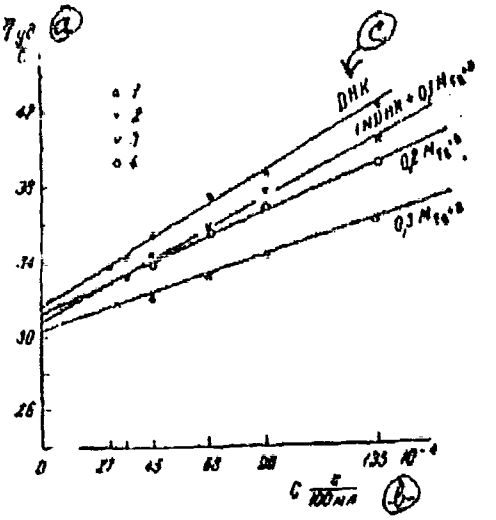

Figure 1. Concentration dependency of the viscosity of a solution of DNA, containing various amounts of $FeCl_3$, on the mole of nucleotides.

1 - 1 M DNA + 0,3 M Fe^{3+};
2 - DNA:
3 - 1 M DNA + 0,1 M Fe^{3+};
4 - 1 M DNA + 0,2 M Fe^{3+}.

a - $\frac{\eta sp}{C}$

b - $C \frac{g}{100\ ml}$

c - DNA

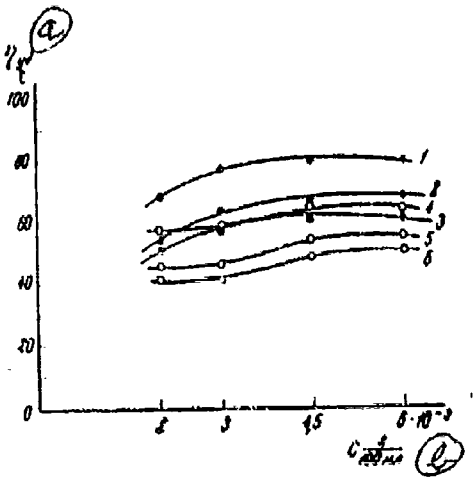

Figure 2. Concentration dependency of the viscosity value of a solution of DNA and a complex of DNA with Fe^{3+} (0.3 M Fe^{3+} per mole of nucleotides)

1, 2 and 3 -- are in regard to a solution of DNA at gradients of 39.6, 45 and 72 sec. respectively;

4, 5 and 6 -- are in regard to a solution of DNA with Fe^{3+} at the same gradients.

a -- $\frac{\eta_{sp}}{C}$

b -- $C \frac{g}{100\ ml}$

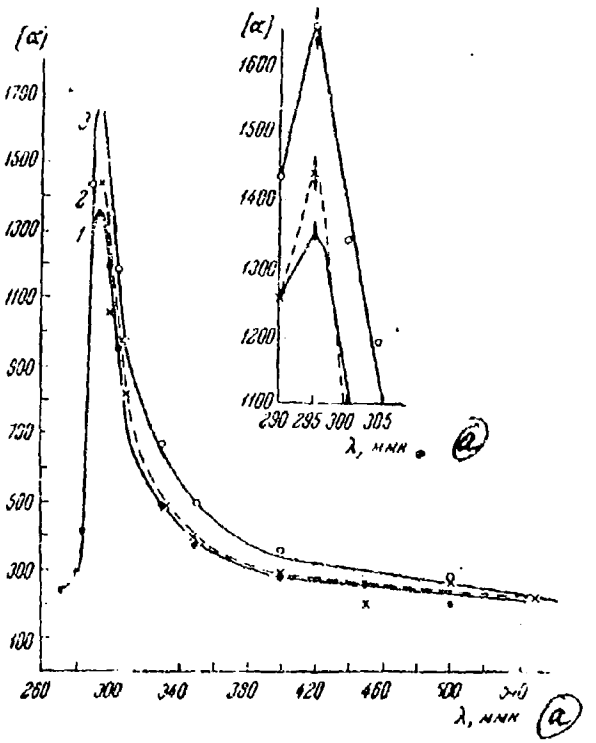

Figure 3. Influence of Fe^{3+} ions on the dispersion curves of optical rotation of solutions of DNA.

1 -- solution of DNA;

2 -- solution of DNA + 1 M Fe^{3+};

3 -- solution of DNA + 0.3 M Fe^{3+}.

a -- λ mμ

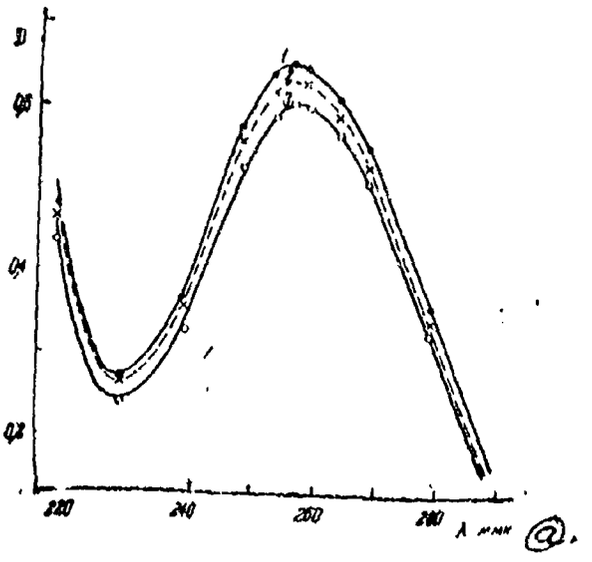

Figure 4. Influence of Fe^{3+} ions on the absorption curves of DNA solutions.

1 -- solution of DNA;

2 -- solution of DNA + 1 M Fe^{3+};

3 -- solution of DNA + 0.3 M Fe^{3+}.

a -- λ mμ

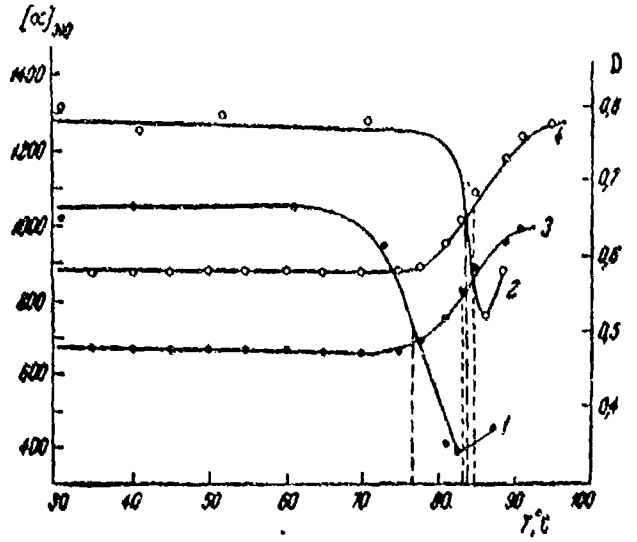

Figure 5. Influence of Fe^{3+} ions on the fusion curves of solutions of DNA, obtained when measuring the optical rotation (1 and 2) and optical density (3 and 4).

1 and 3 -- solution of DNA;

2 and 4 -- solution of DNA + 0.3 M Fe^{3+}

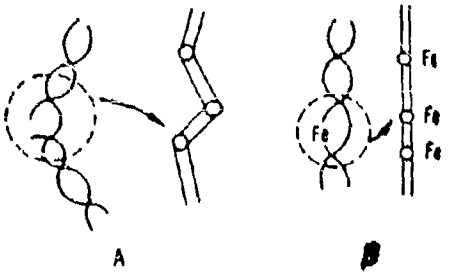

Figure 6. Model of a macromolecule of DNA in the absence of (A) and in the presence of (B) Fe^{3+} ions.